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Headspace solid-phase microextraction of sulphides and disulphides using Carboxen–polydimethylsiloxane fibers in the analysis of wine aroma

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Abstract

Headspace solid-phase microextraction was applied to gas chromatography coupled to flame photometric detection to develop a method for analysing volatile sulphides and disulphides in wine. The Carboxen–polydimethylsiloxane-coated silica fiber was tested and different parameters such as presampling time, ionic strength, stirring, headspace volume, ethanol concentration, time and temperature of extraction were optimized to make extraction as efficient as possible. The optimized conditions enabled limits of detection to be obtained at the ng/l levels. The fiber tested has a strong affinity for the sulphur compounds studied and enables these analytes to be quantitatively determined in wines. The Carboxen–polydimethylsiloxane-coated fiber is more efficient at extracting than fibers such as those which are polydimethylsiloxane-coated and polyacrylate-coated, but its repeatability is worse. The overall process was successfully applied to identify and quantify sulphur compounds in white, red, rosé and vintage wines.

Keywords: Food analysis; Wine; Extraction methods; Sulphides; Disulphides

1. Introduction

Sulphur is a constituent of the amino acids cysteine, cystine, methionine and glutathione. Via Maillard or Strecker reactions, these substances may be degraded into different kinds of sulphur compounds that are present in a great deal of foods and beverages. Many of these analytes, particularly those of high volatility, are powerful odorants with very low sensory thresholds [1]. In wines, the presence of these compounds is usually considered as an off-flavour and, according to the literature, the concentration of these compounds may be affected by the cloudiness of the grape juices [2], the sulphur containing pesticides used [3,4], thermal and photochemical reactions [4,5] and ageing [6].

Since they are commonly found at trace levels, sulphur compounds need to be identified and quantified by such sensitive techniques as gas chromatography (GC) coupled to flame photometric (FPD) or sulfur chemiluminescent detection (SCD). However, before the chromatographic analysis, the analytes have to be preconcentrated. The techniques most widely used for the determination of sulphur compounds, in wines and other alcoholic beverages, are liquid–liquid extraction [7,8], direct static headspace

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(HS) [8,9], static HS with cryogenic trapping [10,11], and dynamic HS (purge and trap) [12,13].

Solid-phase microextraction (SPME) is a recently developed technique [14,15] which uses a polymer-coated silica fiber to extract analytes from a variety of matrices and, directly, transfer them into the injector of a GC system for thermal desorption and analysis.

SPME was originally developed for sampling organic contaminants in water by direct immersion of the fiber into the sample [16], but nowadays it is also applied to the headspace above solid or liquid samples (HS-SPME) in food analysis [17–20]. Polydimethylsiloxane (PDMS) and polyacrylate (PA) fibers have already been used to analyse the volatile sulphur compounds present in wines [21,22]. This study tests the suitability of the Carboxen–polydimethylsiloxane (CAR–PDMS) fiber. This is a new recently marketed fiber which seems to have a strong affinity for the sulphur compounds [23].

2. Experimental

2.1. Chemicals and reagents

The volatile sulphur compounds studied were: dimethyl sulphide (Me_2S) [75-18-3], diethyl sulphide (Et_2S) [352-93-2], methyl-*n*-propyl sulphide (MeSPr) [3877-15-4], methyl thioacetate (MeSAc) [1534-08-3], ethyl thioacetate (EtSAc) [625-60-5], carbon disulphide (CS_2) [75-15-0], dimethyl disulphide (Me_2S_2) [624-92-0], diethyl disulphide (Et_2S_2) [110-81-6]. Ethylmethyl sulphide (MeSEt) [624-89-5] and thiophene [110-02-1] were used as internal standards (I.S.s).

MeSEt and Et_2S_2 were supplied by Aldrich (Beerse, Belgium), MeSAc and EtSAc by Lancaster (Bischheim, France) while the other analytes were supplied by Fluka (Madrid, Spain). Their purity was above 98%. The other auxiliary reagents used in the different studies were supplied by Scharlau (Barcelona, Spain).

An individual standard solution of 2000 mg/l of each sulphide and disulphide was prepared in ethanol and stored in darkness at −10°C. A global standard solution containing all the analytes was prepared with an aliquot of each individual solution and subsequently diluted with ethanol.

Working solutions used in further studies were prepared by diluting different amounts of the global standard solution in a synthetic wine solution [3.5 g/l of L(+)-tartaric acid and 120 ml/l of ethanol in Milli-Q quality water]. In order to obtain a matrix which was as similar as possible to a real wine sample, some other wine volatiles were added: methanol (125 mg/l), ethanal (75 mg/l), ethyl acetate (100 mg/l), isoamyl acetate (10 mg/l), 3-methyl-1-butanol (200 mg/l), 2-methyl-1-butanol (50 mg/l) and potassium metabisulphite (275 mg/l), all of which had a purity above 98%. They were supplied by Aldrich. Finally, the pH was adjusted to 3.5.

2.2. Sample preparation

To avoid loss of the most volatile sulphur compounds, samples were prepared at 4°C. For each SPME analysis, 25 ml of sample (natural or synthetic wine) was pipetted and placed into a 50-ml glass vial with 2.92 g of NaCl (2 *M*) and 0.15 g of EDTA [24]. Each sample was spiked with MeSEt and thiophene, to give a final concentration of 10 μg/l and 2.5 μg/l, respectively. The vial was tightly capped with a PTFE-faced silicone septum and shaken. Following previous works [10,21], two internal standards were used and the most suitable one was chosen to quantify each analyte at the concentration level found in wines.

2.3. Headspace and SPME

The SPME device and CAR–PDMS (75 μm) fibers used in this study were purchased from Supelco (Bellefonte, PA, USA). The fibers were conditioned by inserting them into the GC system injector at 280°C for 30 min and they were immediately used to prevent contamination.

Before the extraction with the fiber, the sample vials were equilibrated for 30 min at 25°C. Afterwards, the stainless steel needle in which the fiber is housed was pushed through the vial septum, allowing the fiber to be exposed to the headspace of the sample for 30 min. Then, the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 300°C for 1 min.

The ULC (univariate linear calibration) computer programme [33] was used to calculate, by linear least-squares regression, the slope and intercept with the correlation coefficient (r^2).

2.4. Chromatography

The analyses were made on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with an HP Model 19256A flame photometric detection (FPD) system operated in the sulphur mode. The injection was made in the splitless mode for 1 min at 300°C using an inlet of 0.75 mm I.D. which improved the GC resolution. The temperature of the detector was 200°C and it was fed with 75 ml/min of hydrogen, 86 ml/min of synthetic air and 57 ml/min of helium as auxiliary gas. The detector signals were sent to an HP Chemstation, where they were collected, integrated and recorded.

Compounds were identified by comparing GC retention times using two different chromatographic columns. The separations were performed using an SPB-1 column (30 m×0.32 mm I.D., 4 μm) and the oven temperature was programmed as follows: 50°C (8 min), 15°C/min to 150°C, 40°C/min to 280°C (5 min). Helium was used as carrier gas with a flow-rate of 1.2 ml/min. The column used to check the identity of the analytes in real samples was an HP-Innowax column (50 m×0.2 mm I.D., 0.2 μm) with an oven temperature programme of 40°C (8 min), 50°C/min to 220°C (10 min). The carrier gas was helium with a flow-rate of 0.4 ml/min.

To determine the identity of other wine volatiles which were also extracted by the fiber, as well as the sulphur compounds, a Hewlett-Packard 5890 (series II) gas chromatograph equipped with an HP-5972 mass-selective detector was used. Injection was made in the same way as in FPD. The detector operated in electron impact mode (70 eV) at 280°C. Detection was in the scan mode between 30 and 300 u.

3. Results and discussion

The CAR–PDMS (75 μm) fiber is coated with porous carbon which makes it possible to use SPME to analyse volatile analytes at trace levels. This fiber has been used to extract volatile organic compounds from water and air [23,25], and sulphur gases [23]. In this study, this new fiber was used to analyse eight volatile sulphur compounds commonly found in wines.

In the analytical method developed, several variables before the sample injection were optimized. The experiments were carried out with five samples of synthetic wine spiked with 2.5 μg/l of each sulphide (RSR) and 1.25 μg/l of each disulphide (RSSR). MeSEt and thiophene were used as internal standards at concentration levels of 10 μg/l and 2.5 μg/l, respectively.

Previous studies have reported that SPME analysis is not influenced by air, water or soil matrices [26–28]. However, in alcoholic beverages, ethanol affects the extraction [21,29,30]. Furthermore, due to the high extraction efficiency of the fiber, we found that some wine volatiles may interfere in the extraction of the sulphur compounds studied. The extractions of these sulphur compounds in spiked real wines and different synthetic wines were compared and the results showed that the wine matrix influenced the extraction, as can be seen in Fig. 1. This figure shows the relative responses of the MeSPr/MeSEt peak area ratio (as RSR compound) and the CS_2/MeSEt peak area ratio (as RSSR compound) after SPME at different concentrations in a white wine, a red wine, a synthetic wine without volatiles (SW) and a synthetic wine spiked with some volatiles (SWV). It can be seen that the chromatographic response in real wines is close to the response in SWV, whereas the response of the SW sample is much lower. This difference is more evident in the RSSR because its higher sulphur content makes it more sensitive to FPD.

The volatiles added to obtain the SWV matrix were ethanol, methanol, ethanal, ethyl acetate, isoamyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol and sulphur dioxide. These were selected because of their high SPME efficiency which we found by injecting the headspace of different wines into the GC–mass spectrometry (MS) system. These results agree with the ones obtained by other authors [31]. So, to optimize the SPME parameters, a synthetic wine that contained all of these volatiles was used.

Since the samples had to be prepared at 4°C, the analytes needed some time to reach the gas–liquid equilibria at room temperature. This time was varied

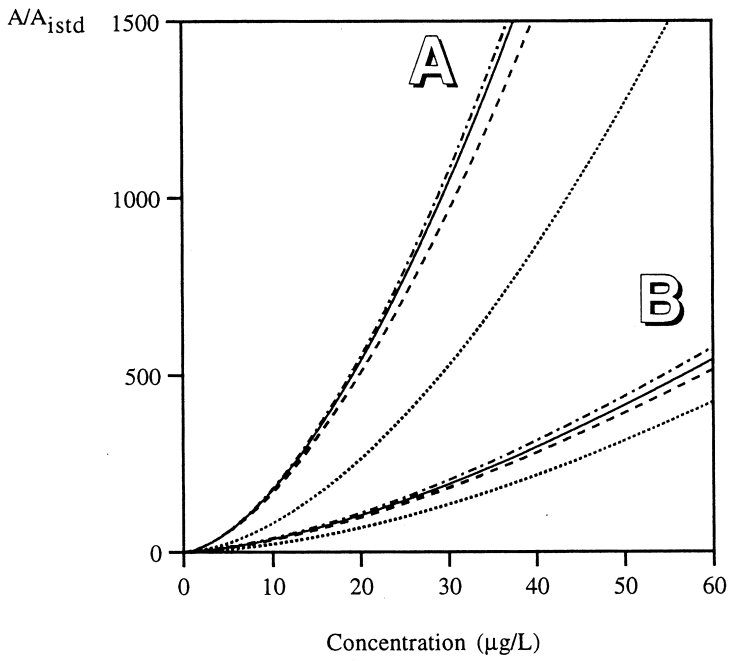

Fig. 1. Relative response after SPME of CS_2 (A) and MeSPr (B) in SW (· · ·), SWV (———), red wine (- - -) and white wine (-·-·-·).

and similar results were obtained with 5, 15, 30 and 60 min, both with and without stirring. We chose a time of 30 min without stirring to coincide with chromatographic run times.

High temperatures are needed to release analytes into the headspace, allowing a better extraction during the SPME sampling. However, SPME involves an exothermic process [28] and the extraction of analyte by the fiber coating decreases as the temperature rises. To check this opposing effect, temperatures from 25–40°C were tested and compared. The results showed that recoveries decreased as the temperature increased, so the value of 25°C was chosen.

Stirring is another parameter that influences extraction, because it causes turbulence in the liquid and gaseous phases [14,15]. Constant stirring was applied in all the SPME experiments because almost all the analytes doubled their detection signal.

HS-SMPE is based on the equilibrium of analytes among the three system phases: the coated fiber, the headspace and the sample solution. The limiting step in this extraction is the diffusion of the analytes through the system [32] and, because of this, the extraction time should be optimized. In this work, periods of 5, 15, 30 and 60 min were tested. The results showed that a minimum of 15 min is required to reach equilibrium. Beyond this time, the amount of analyte extracted vs. the extraction time becomes constant. Since the chromatographic run time was 30 min, for practical reasons, the time fixed for the extraction was also 30 min.

Adding salt to the sample increases the extraction efficiency because the ionic strength clearly affects the amount of analytes released into the headspace and, therefore, into the coated fiber. Ionic strengths between 0 and 6 *M* of NaCl were tested and compared. The response rises slightly when the salt concentration increased to 2 *M* and becomes constant at higher concentrations. So, 2 *M* was the salt concentration fixed for subsequent analysis.

The ethanol concentration was also studied. Since ethanol is one of the major constituents of wines (11–14%) and is extracted by the fiber, it competes with the sulphur compounds, which are found in much smaller concentrations. The data obtained show that the higher the ethanol concentration, the lower the extraction efficiency. These results agree

with those published by other authors [21,29,30]. Dilution of samples is recommended in order to decrease the ethanol content. Since it is not possible to dilute the samples without affecting the concentration of the sulphur compound traces, the reproducibility of the method was assured by adjusting the samples to 12% ethanol, either by dilution with water or by addition of ethanol.

Finally, different volumes (10, 20 and 25 ml) of one sample were placed into a 50-ml sampling vial and extracted for 30 min, in order to check if the equilibration time could be reduced by using a smaller headspace [21]. The results showed that extraction increases as the headspace volume decreases. No more than 25 ml of sample was used so that the fiber did not touch the liquid.

Fig. 2 shows the chromatogram obtained when a sample of synthetic wine (SWV) spiked with 2.5 μg/l of RSR and 1.25 μg/l of RSSR was extracted and injected under the conditions described above. The resolution between all the peaks was good.

Once the preliminary studies had been completed and the extraction parameters optimized, the feasibility of the HS-SPME was investigated. The method was assessed by estimating the repeatability, reproducibility, linear range and the limits of detection and quantification.

The repeatability of the new fiber was low, as had been reported in previous studies of volatile organic compounds in water and air [25]. Table 1 shows the

Table 1
[Sulphur compound/MeSEt] peak area ratios with their RSDs (in %) obtained with three different fibers coated with CAR–PDMS

Compound	Fiber 1	Fiber 2	Fiber 3
MeSMe	0.07 (7.43)	0.47 (8.73)	0.50 (11.64)
CS_2	2.49 (13.21)	1.17 (9.38)	1.75 (5.83)
MeSEt	1.00 (–)	1.00 (–)	1.00 (–)
Thiophene	15.68 (8.48)	14.20 (12.34)	16.20 (18.30)
MeSAc	0.18 (6.50)	0.15 (2.37)	0.21 (17.03)
EtSEt	1.35 (8.62)	0.93 (3.15)	1.00 (7.21)
MeSPr	2.58 (12.19)	1.84 (4.03)	1.95 (4.21)
MeSSMe	2.60 (5.29)	1.02 (4.26)	1.27 (1.94)
EtSAc	0.84 (6.98)	0.42 (9.40)	0.49 (5.12)
EtSSEt	63.0 (16.26)	21.30 (4.07)	37.27 (11.27)

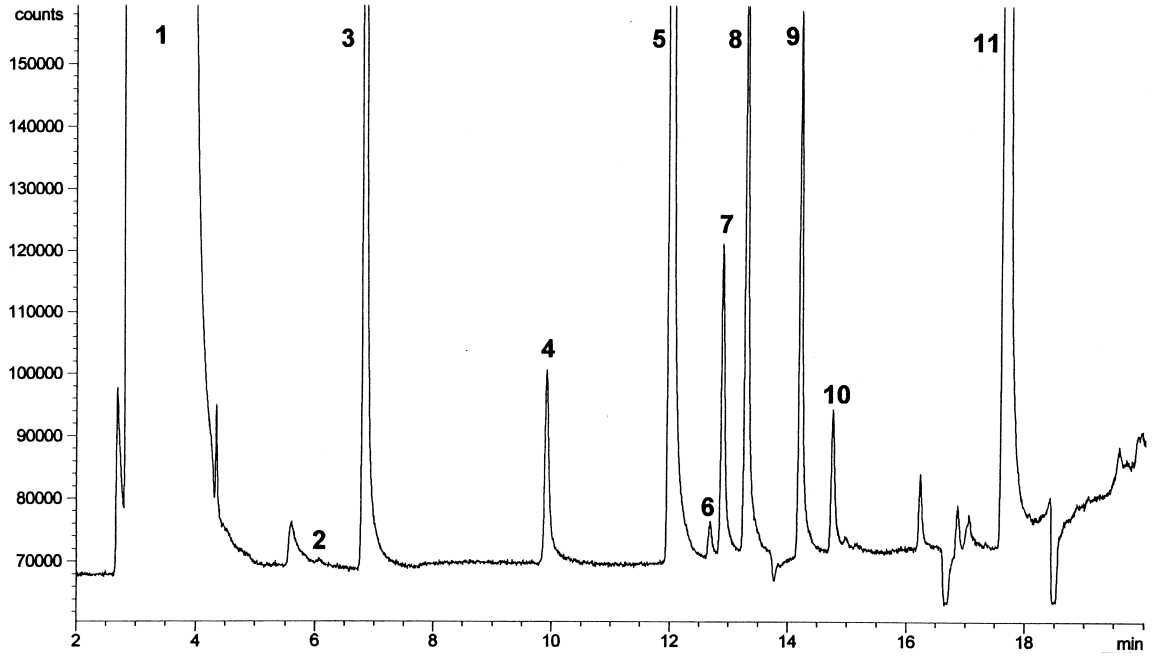

Fig. 2. Chromatographic response of a standard solution of sulphur compounds in synthetic wine (SWV) after microextraction with Carboxen–polydimethylsiloxane fiber. 1=Sulphur dioxide, 2=dimethyl sulphide, 3=carbon disulphide, 4=ethylmethyl sulphide (I.S.), 5=thiophene (I.S.), 6=methyl thioacetate, 7=diethyl sulphide, 8=methylpropyl sulphide, 9=dimethyl disulphide, 10=ethyl thioacetate, 11=diethyl disulphide.

Table 2
Limits of detection of the method (HS-SPME) using different fibers

Compound	LOD (mg/l)		
	PDMS	PA	CAR–PDMS
MeSMe	2.00	3.00	5.00
CS_2	0.40	0.50	0.06
MeSAc	1.50	2.00	1.00
EtSEt	0.25	0.50	0.10
MeSPr	0.25	0.50	0.08
MeSSMe	0.20	0.20	0.06
EtSAc	1.25	1.25	0.25
EtSSEt	0.05	0.10	0.03

[sulphur compound/MeSEt] peak area ratios, with their relative standard deviation (RSD), of the same sample (synthetic wine fortified with 2 μg/l of RSR and 1 μg/l of RSSR) extracted with three different fibers coated with CAR–PDMS. The RSD obtained for each fiber ranges between 2–20%, while the values obtained with PA and PDMS fibers ranged between 5–9% [21] for the same compounds. Considerable differences were also observed among the responses of the various CAR–PDMS fibers. As a result, the overall experiments should be performed with a single fiber and, if more than one is used, the calibration graphs must be recalculated for each fiber.

The calibration graph of the SPME method was obtained from three replicates of the SWV sample spiked with seven different concentrations of the analytes. The FPD response is a power function, so the calibration graphs of the sulphur compounds were constructed by plotting the log [sulphur compound/I.S.] peak area ratios against the log [sulphur compound/I.S.] concentration ratios. The range of linearity studied was between 0.25–80 μg/l for the RSR and 0.125–40 μg/l for the RSSR. In all cases, the correlation coefficient was good ($r^2 > 0.99$).

As has been mentioned above, the CAR–PDMS fiber is highly efficient at extracting the sulphur compounds studied and gives very low limits of detection (LODs). The LOD for each analyte was calculated from the amount of sulphur compounds required to give a $S/N=3$, after applying the HS-SPME method to a fortified synthetic wine (SWV). Table 2 compares the LOD obtained with the fibers coated with PDMS and PA (used in previous work [21,22,22]), and the ones coated with CAR–PDMS. Only the Me_2S gives higher LOD with the new fiber, because of the peak broadening caused by the anomalous desorption of this very volatile compound.

The recovery of the HS-SPME procedure was determined by a standard addition technique with white and red wines. The analytes were added to wines at three different concentration levels: 0.25 μg/l and 0.5 μg/l (first level), 1.25 μg/l and 2.5 μg/l (second level) and, finally, 12.5 μg/l and 25 μg/l (third level) for disulphides and sulphides, respectively. Samples from each level were extracted three times using two different fibers and the results are shown in Table 3. As can be seen, all the recoveries are close to 100%, and both fibers give similar results for both types of wine.

The chromatogram of a typical sample of wine

Table 3
Recovery percentages and relative standard deviations (in parentheses)

Compound	Fiber 1						Fiber 2					
	White wine			Red wine			White wine			Red wine		
	1st level	2nd level	3rd level	1st level	2nd level	3rd level	1st level	2nd level	3rd level	1st level	2nd level	3rd level
MeSMe	– (–)	– (–)	98 (14)	– (–)	– (–)	99 (9)	– (–)	– (–)	96 (16)	– (–)	– (–)	101 (8)
CS_2	125 (29)	101 (9)	109 (28)	98 (10)	104 (1)	89 (5)	131 (9)	107 (9)	110 (28)	96 (11)	100 (1)	117 (9)
MeSAc	90 (5)	103 (8)	100 (26)	112 (28)	107 (18)	112 (9)	98 (11)	109 (8)	118 (9)	114 (11)	98 (8)	119 (18)
EtSEt	101 (24)	99 (3)	95 (21)	97 (19)	105 (5)	95 (14)	109 (17)	96 (17)	82 (4)	119 (17)	107 (7)	118 (19)
MeSPr	95 (15)	100 (7)	98 (21)	102 (2)	104 (5)	95 (12)	97 (2)	115 (7)	82 (7)	107 (8)	102 (15)	121 (23)
MeSSMe	102 (31)	101 (18)	103 (16)	91 (31)	104 (4)	98 (12)	94 (28)	99 (16)	103 (27)	118 (7)	100 (3)	113 (19)
EtSAc	96 (11)	110 (2)	104 (23)	116 (16)	115 (12)	102 (11)	110 (20)	100 (22)	102 (25)	115 (25)	99 (8)	114 (19)
EtSSEt	85 (23)	89 (25)	104 (28)	100 (2)	97 (2)	197 (30)	105 (29)	93 (8)	119 (26)	122 (29)	97 (16)	113 (13)

Conditions given in Section 2.

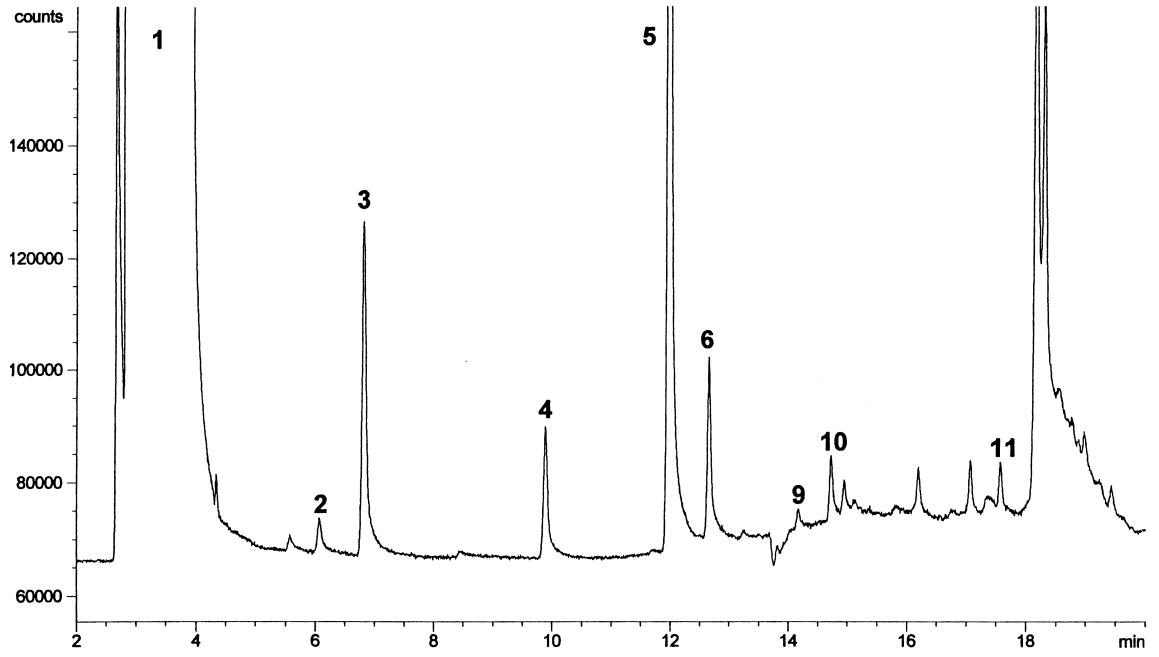

Fig. 3. Chromatographic response of a real sample of wine analysed using the proposed procedure. Peak identification numbers are the same as in Fig. 2.

(Fig. 3) shows that the sulphur compound peaks were well resolved, although a high SO_2 peak appeared at the beginning of the chromatogram. As can be seen, other sulphur compounds, which are the subject of further studies, appear in the chromatogram.

The method was used to determine the sulphur compound contents of different red, white, rosé and vintage wines. The ranges (μg/l) of the sulphur compounds studied were: Me_2S (not detected–60), CS_2 (0.6–18), MeSAc (not detected–12), Et_2S (not detected–2), MeSPr (not detected–0.75), Me_2S_2 (not detected–4), EtSAc (not detected–3), Et_2S_2 (not detected–1). These values agree with the results found in the literature [8,10,11,21].

4. Conclusions

HS-SPME using the CAR–PDMS fiber is a good technique for determining the sulphur compound content in wines at ng/l levels. This simple, solventless and fast technique enables volatile and less volatile sulphur compounds to be determined simultaneously without analyte loss. CAR–PDMS makes the technique more sensitive than when other coated fibers are used, but matrix interferences have to be taken into account, and a specific synthetic wine must be used to validate the method.

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